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Synthesis and Evaluation of 1,2,4-oxadiazolidinones: The Search for Potential non-β-lactam β-lactamase Inhibitors.

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Synthesis of 1,2,4-oxadiazolidinone derivatives:

The Search for Potential Non-β-lactam β-Lactamases Inhibitors

presented by

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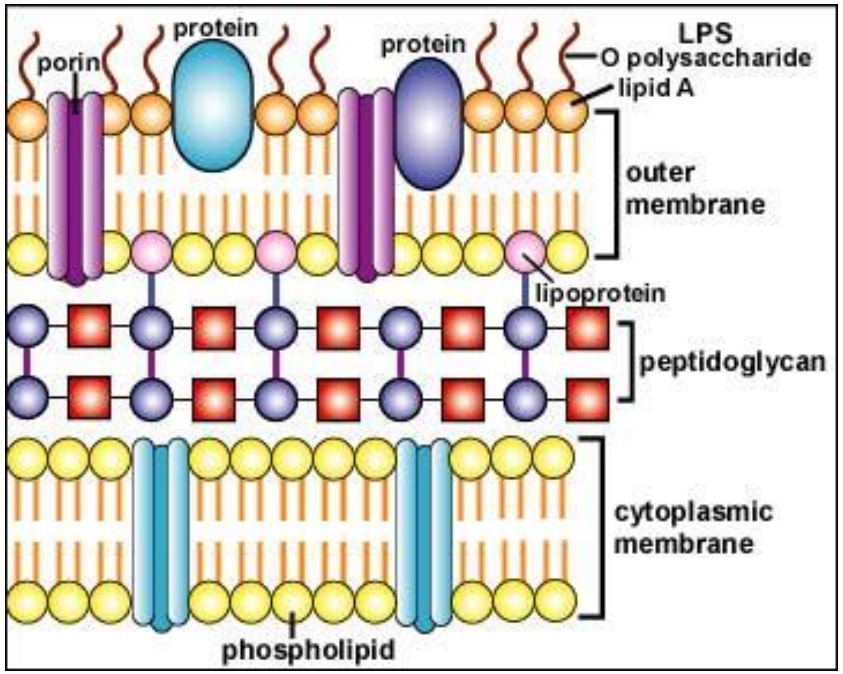
Outline

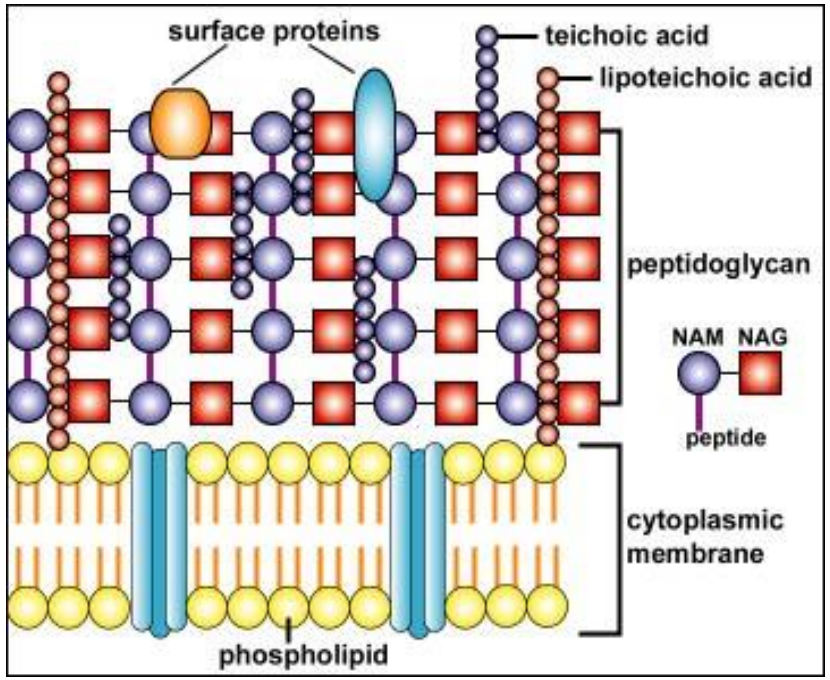
- Introduction
- Research objectives
- Bacteria cell wall structure
- β-lactam antibiotic drugs
- Non β-lactam β-lactamase inhibitor
- Synthesis, characterization, and biological activites of 1,2,4-oxadiazolidinone analogs
- Conclusion

Research Objectives

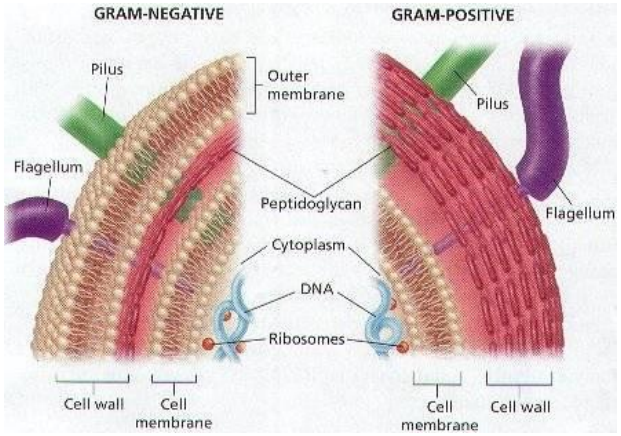
- To synthesize a 1,2,4-oxadiazolidinone derivatives via 1,3-dipolar cycloaddition of nitrones with substituted isocyanates.
- To evaluate the biological significance of the synthesized inhibitors.
- To improve or restore the potency of antibiotic agents that lost their effectiveness due to continuous evolution of bacteria's β-lactamases.

Bacterial cell wall





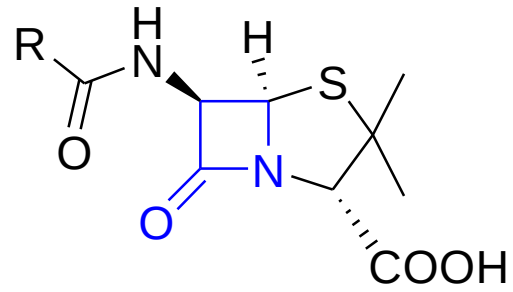
Gram Negative



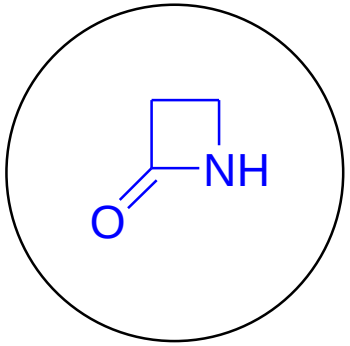
Gram Positive

Figure 1: A section of Gram-negative and Gram-positive cell wall

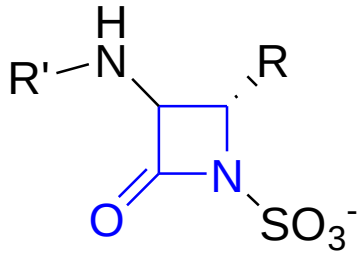
β-lactam antibiotic drugs



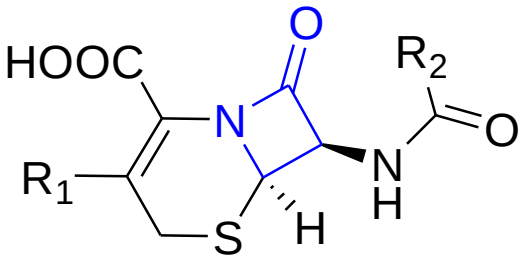
Penicillin



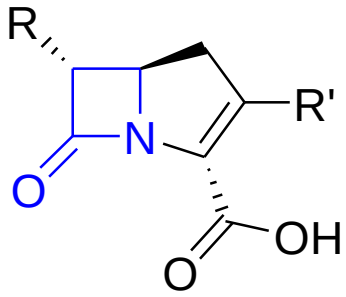
ß-Lactam Ring



Monobactam



Cephalosporin



Carbapenems

Figure 2: Some β-lactam Antibiotics

Bacteria' β-lactamases

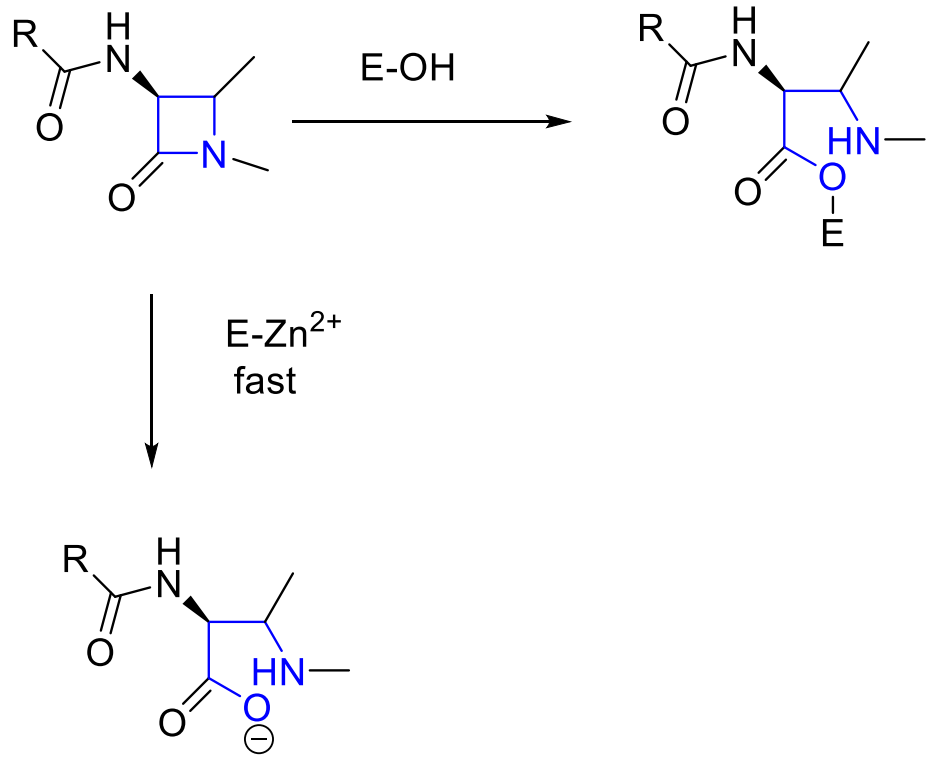
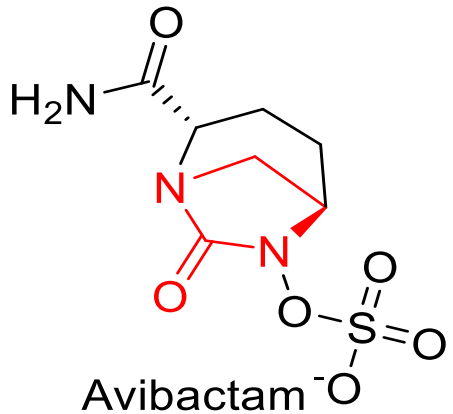
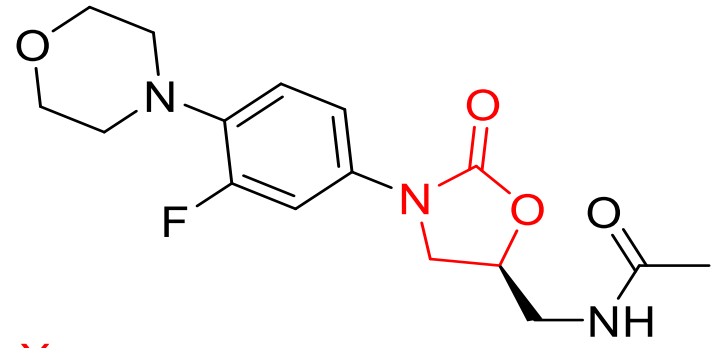


Figure 3: Mechanism of resistance to antibiotics.

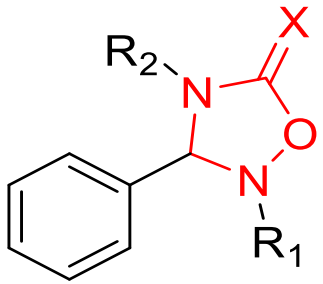
Justification of 1,2,4-oxadiazolidin-5-one



Avibactam



Linezolid



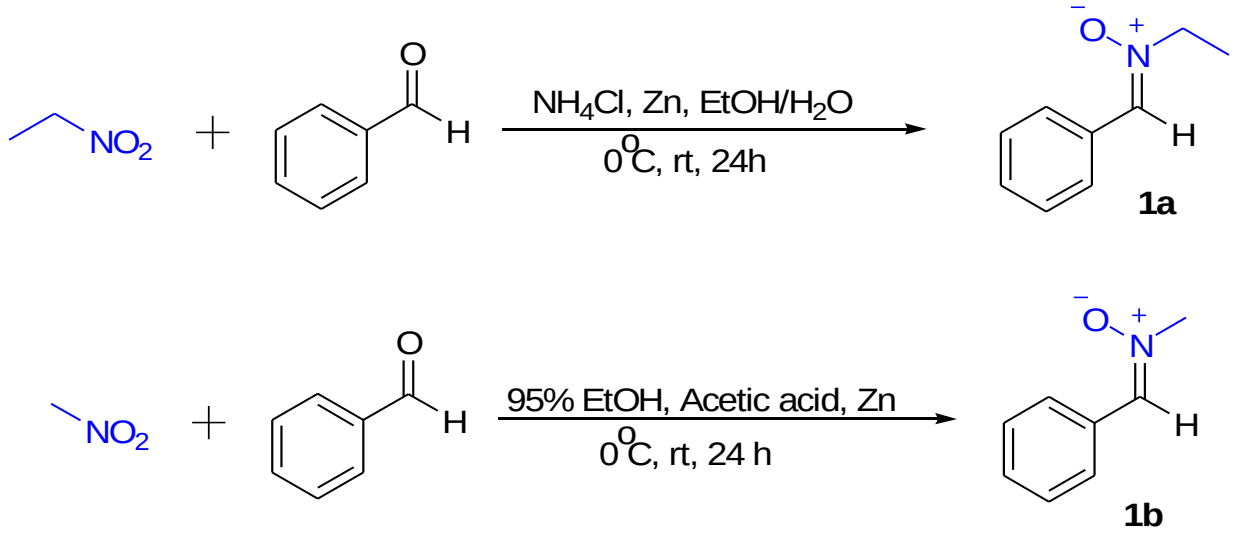
Oxadiazolidinone scaffold

$R_1 = CH_3, C_2H_5$
$R_2 = Ph, C_2CO_2C_2H_5, C_2CO_2H$
$X = O, S$

Figure 4: Comparison of compound of interest with Avibactam and linezolid

https://pubchem.ncbi.nlm.nih.gov/compound/9835049
www.idstewardship.com/drugs/ceftazidime-avibactam
http://www.avalonpharmacy.com/product/zyvox-linezolid/

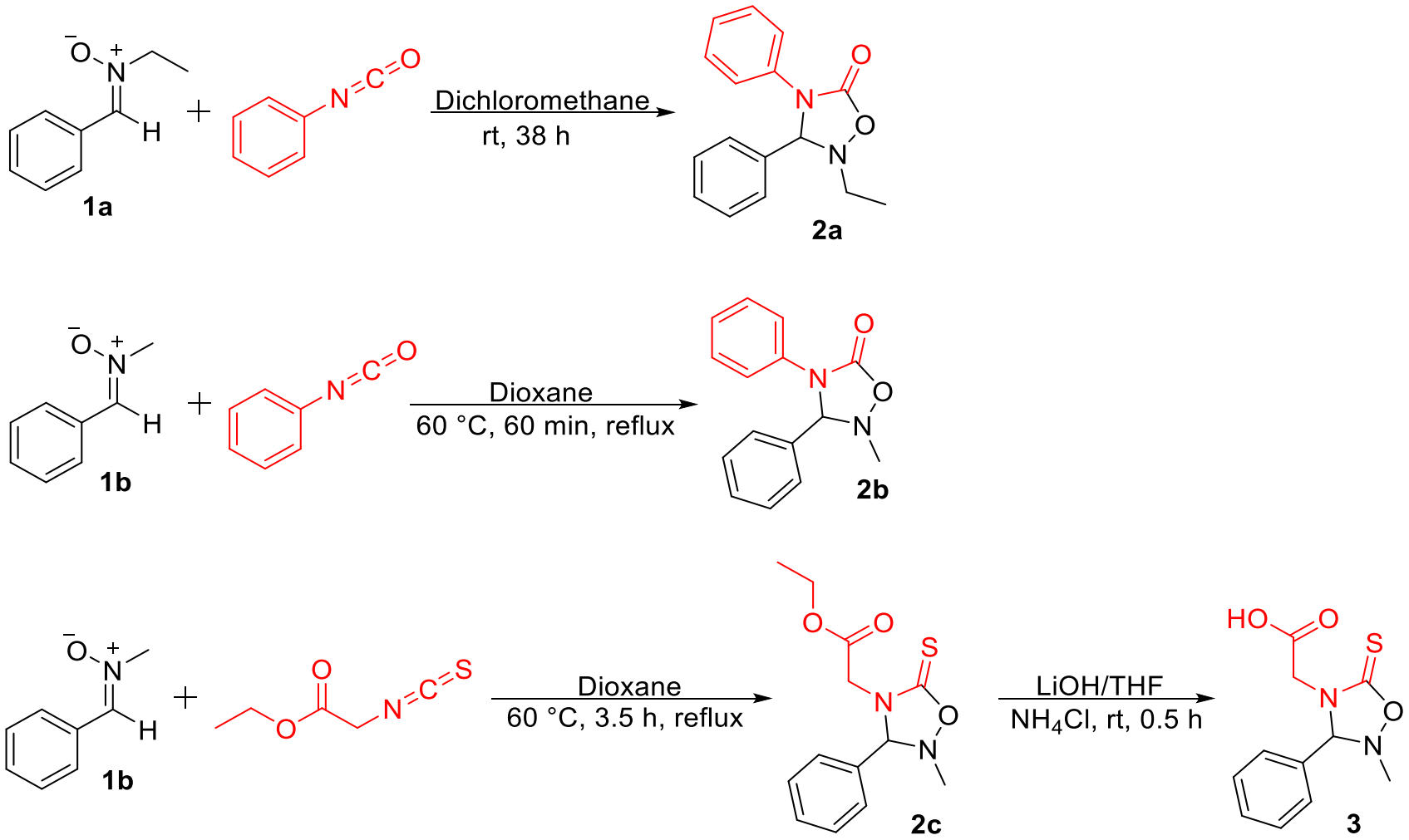
Synthesis of nitrones



Scheme 1: Reaction of nitro compounds with benzaldehyde.

Ritter, T., Carreira, E. M. *Angew. Chem., Int. Ed.* **2005,** *44* (6), 936-938

1,3-dipolar cycloaddition reaction



Scheme 2: Reaction of nitrones with various isocyanates

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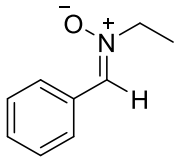
CHARACTERIZATION AND BIOLOGICAL ACTIVITIES



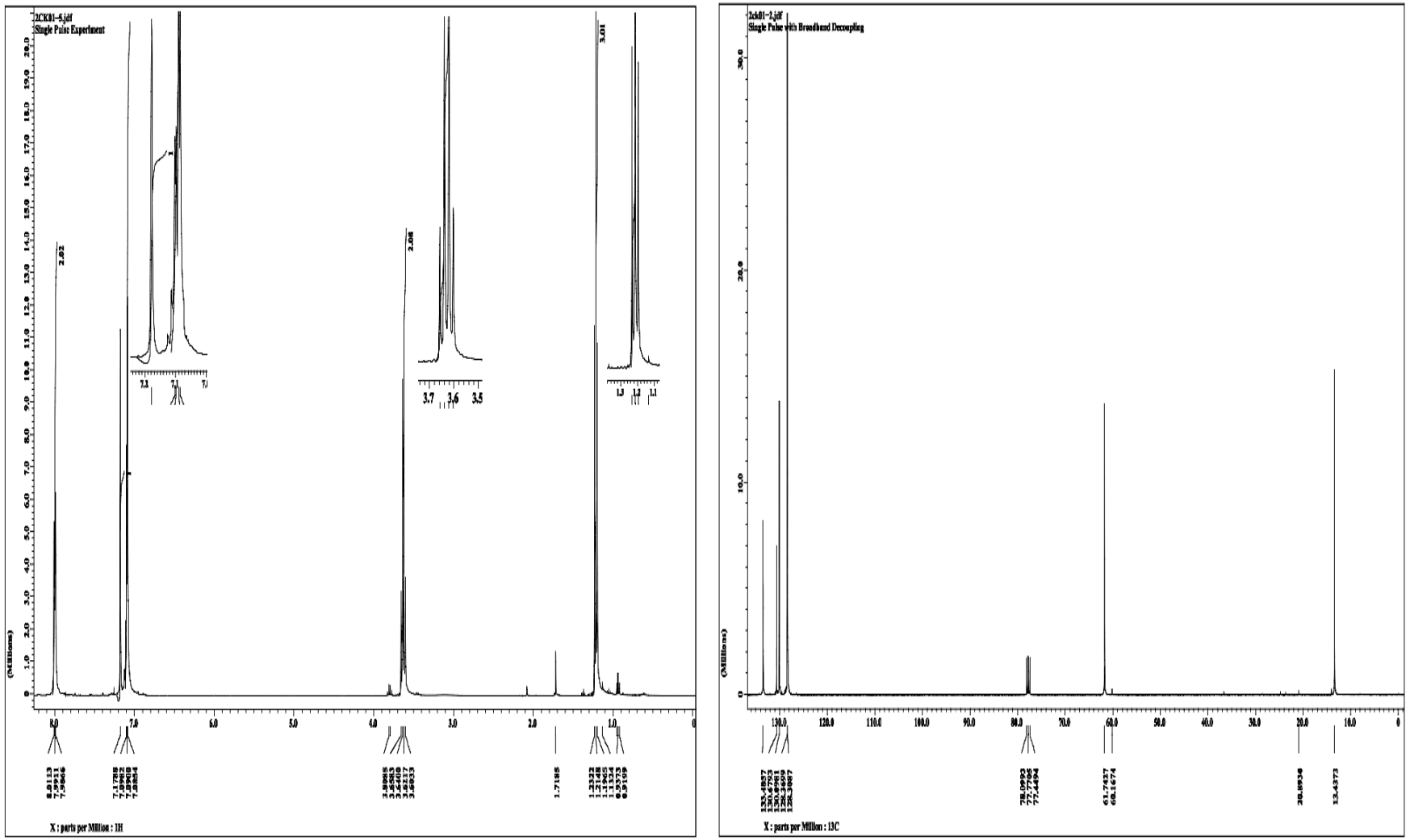


Figure 5: ^{1}H & ^{13}C NMR spectra of Nitrone **1a**

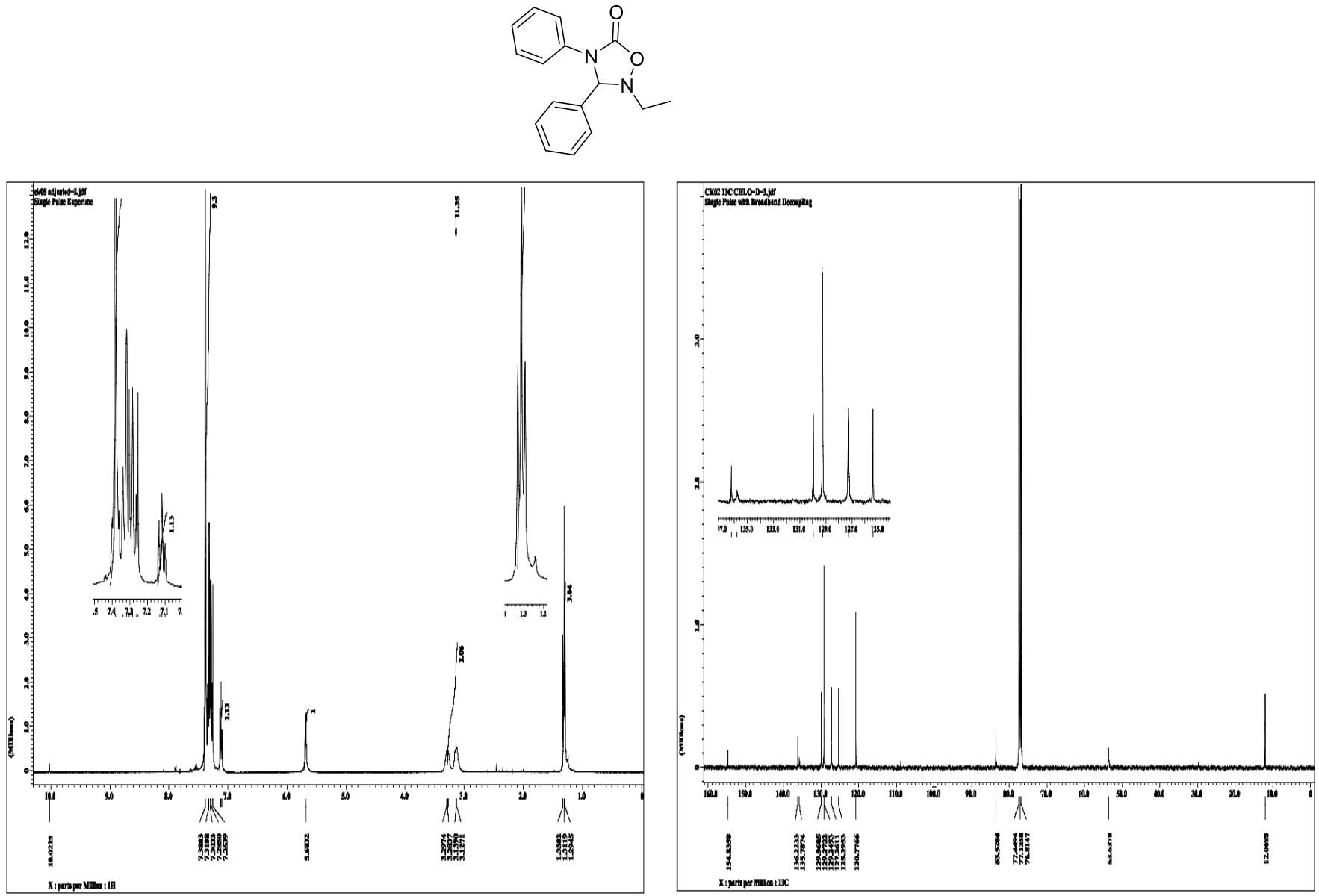


Figure 6: ^{1}H & ^{13}C NMR spectra of oxadiazolidinone **2a**

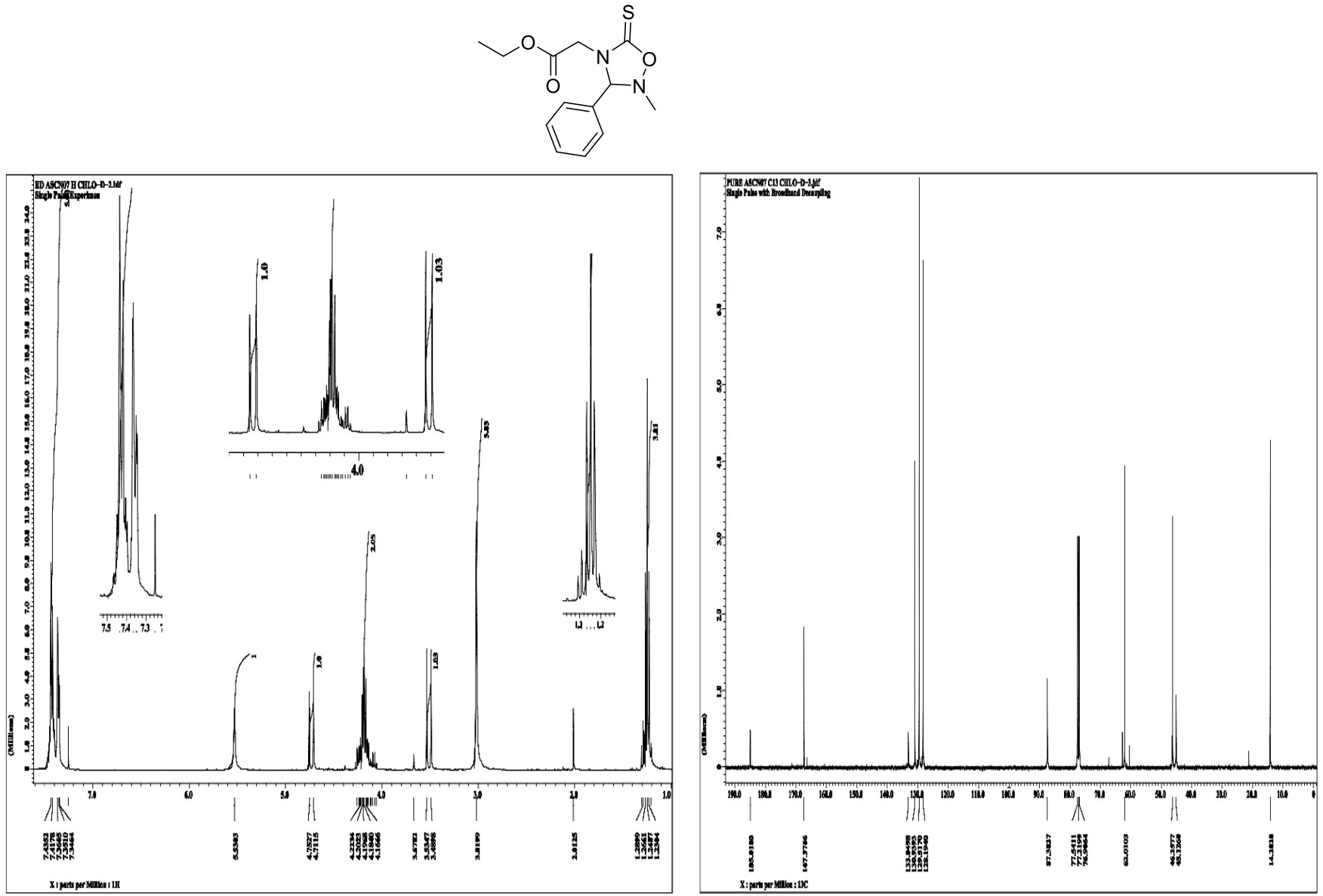
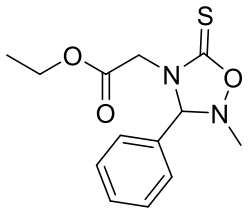


Figure 7: ^{1}H & ^{13}C NMR spectra of compound **2c**

# of Peaks	436
Raw Spectrum	11.139 (scan: 878)
Background	No Background Spectrum
Exact mass	280



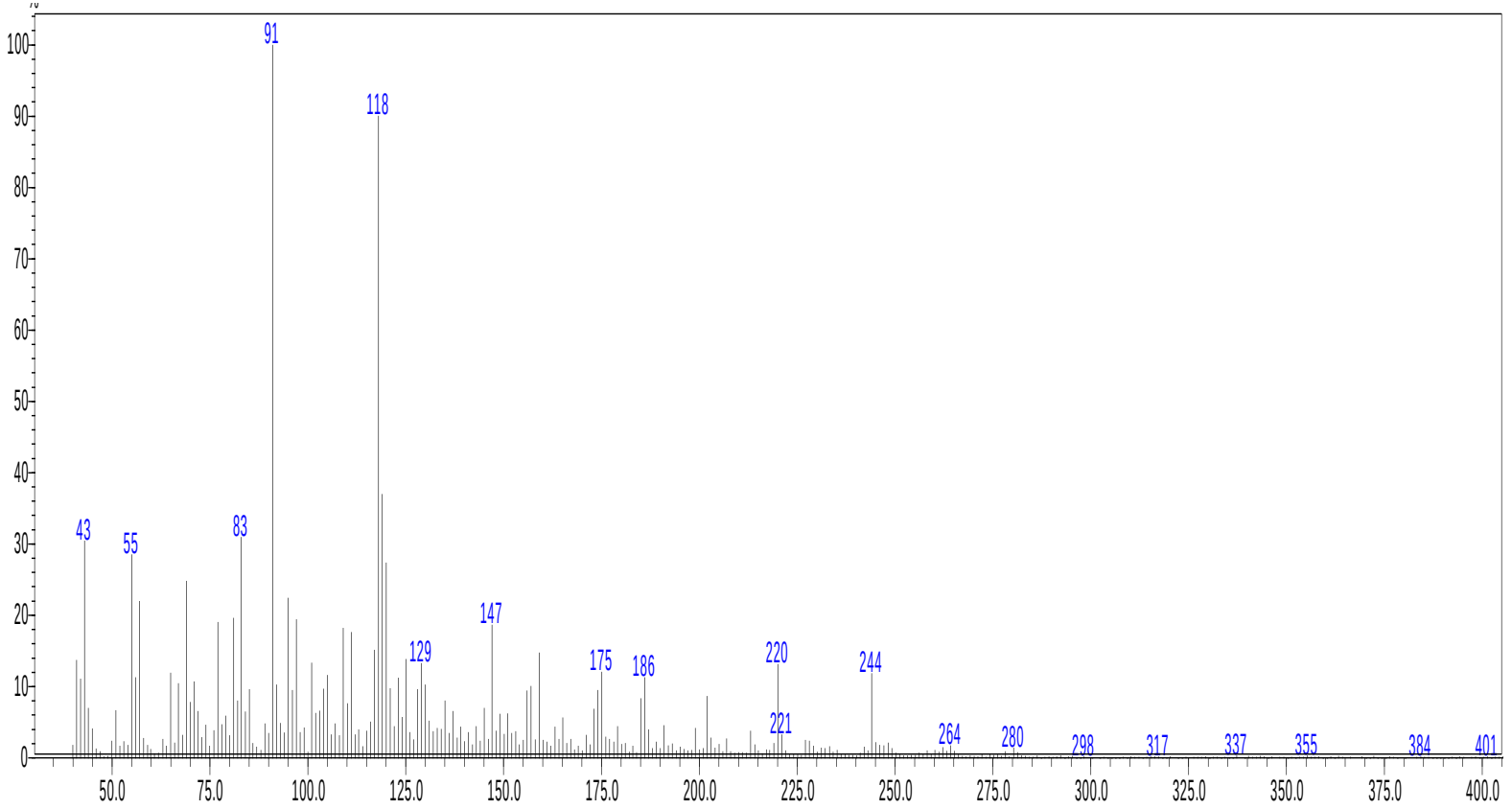


Figure 8: GC-MS spectrum of compound **2c**

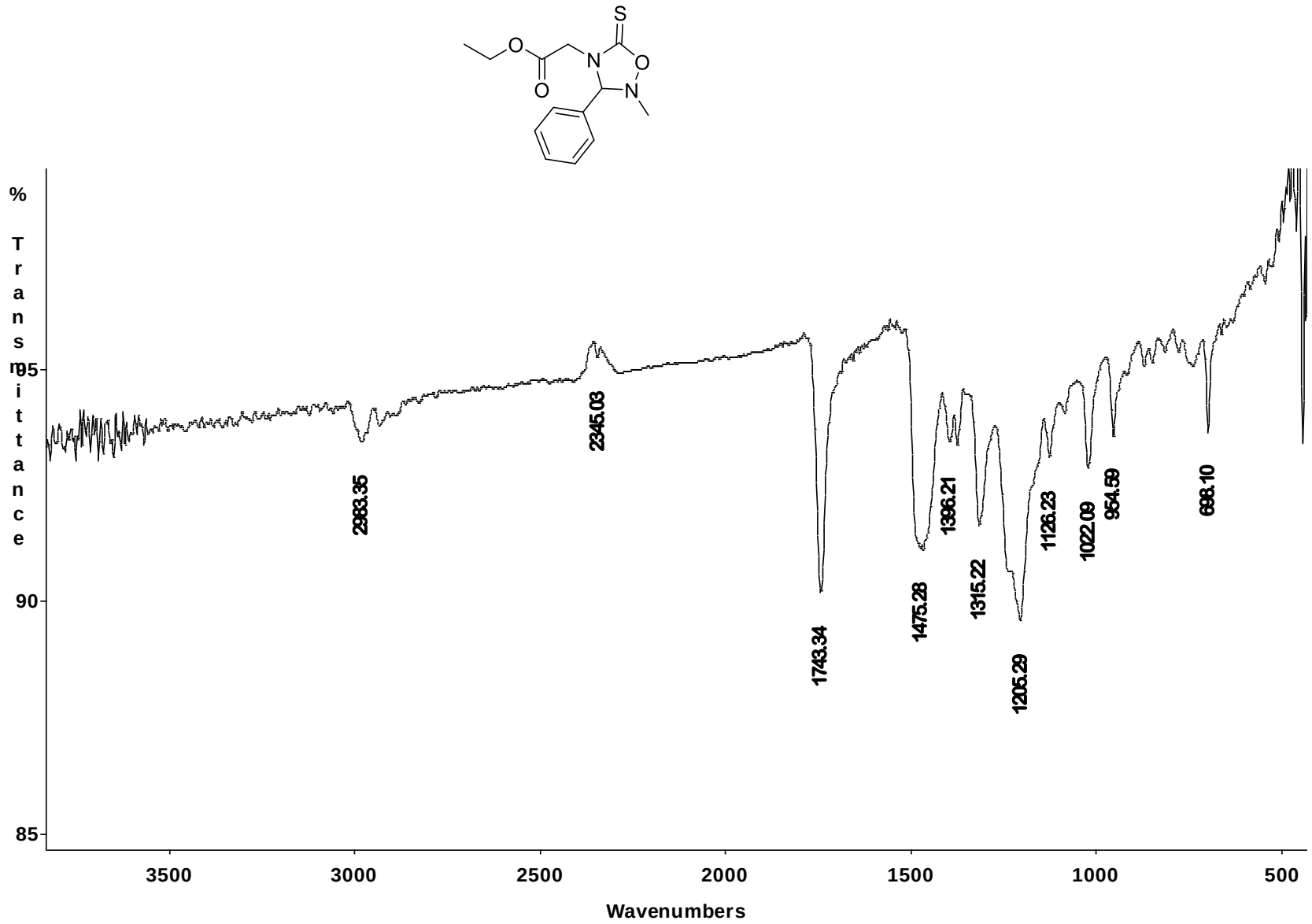


Figure 9: IR spectra of compound **2c**

Demonstration of cycloaddition

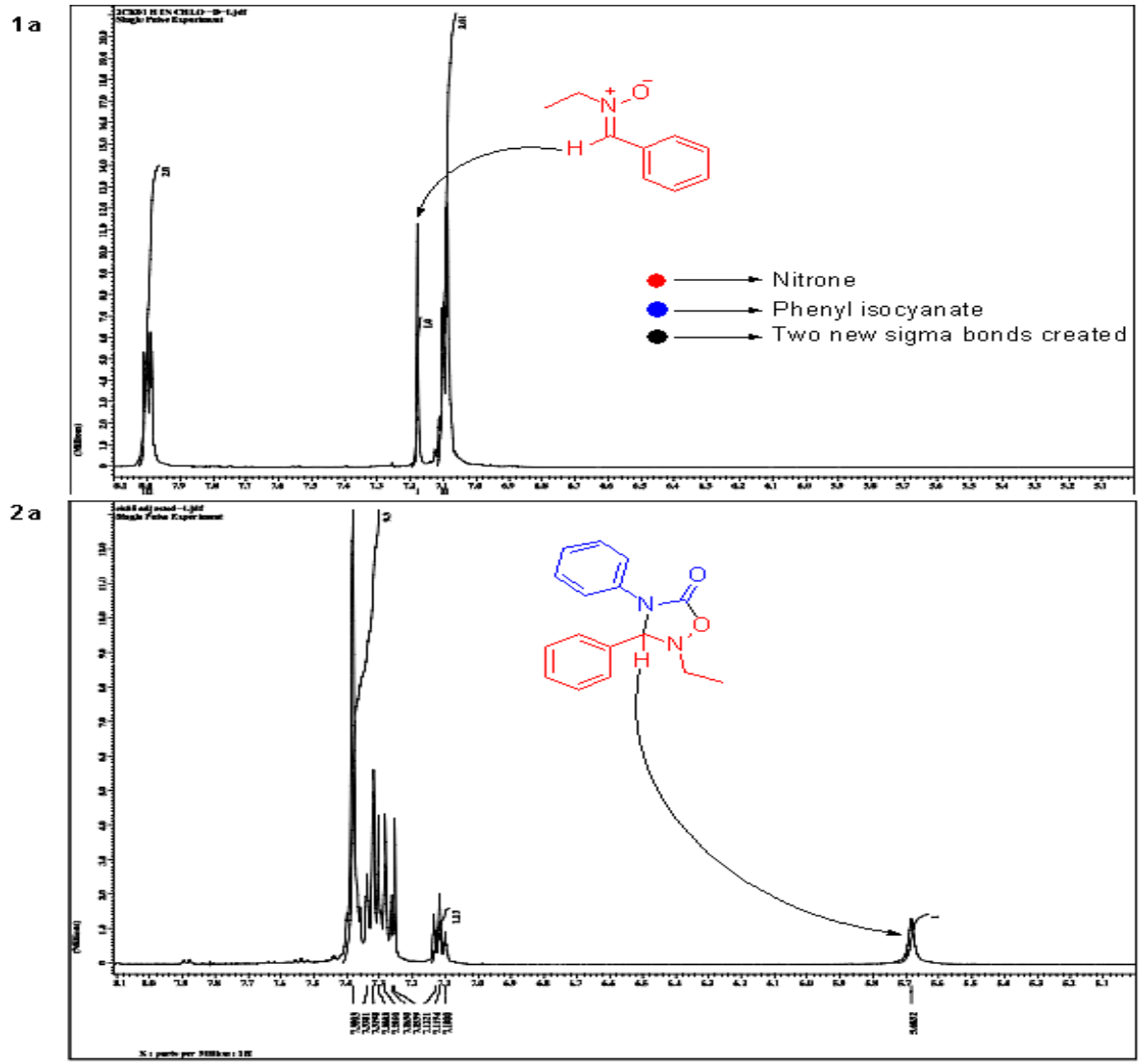
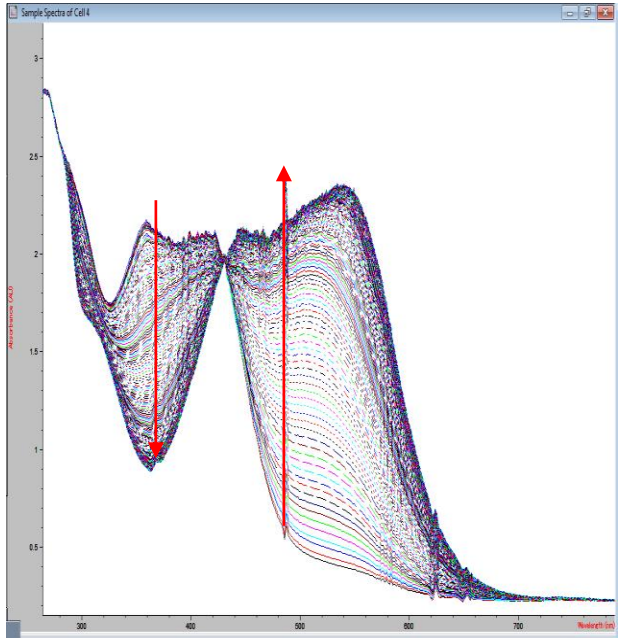
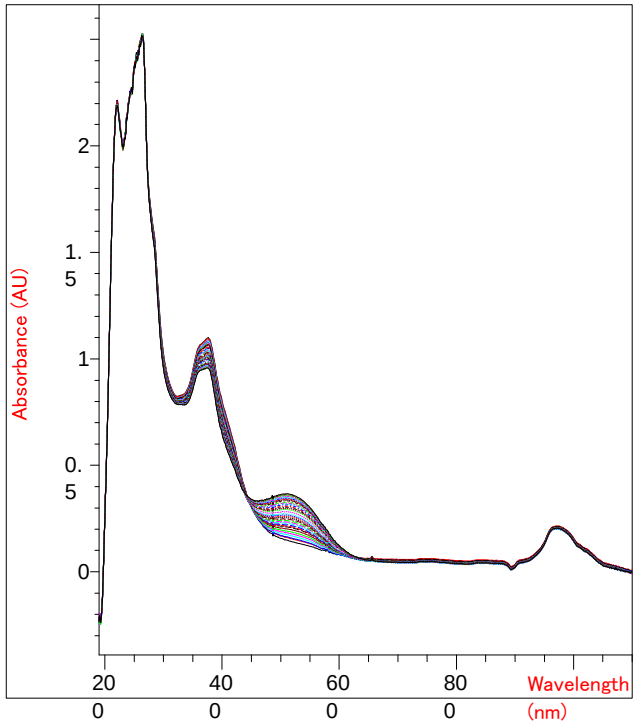


Figure 10: ^{1}H NMR spectra of **1a** and corresponding oxadiazolidinone **2a**

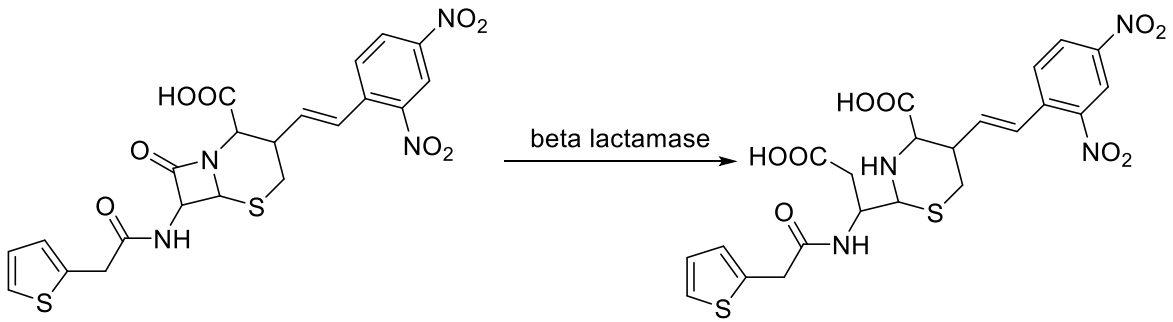
Table 1: The cytotoxicity essay (%) of **2a** on relative control (100%) against IGROV1, OVCAR-4, HS 578T, BT-549, A498, UO-31, UACC-62, SK-MEL-28

Cancer types	Cell line	Growth %	% Inhibition
Ovarian Cancer	IGROV1	95.59	4.41
	OVCAR-4	95.84	4.16
Breast Cancer	HS 578T	93.06	6.94
	BT-549	99.50	0.50
Renal Cancer	A498	82.27	17.73
	UO-31	86.72	13.33
Melanoma	UACC-62	93.93	6.07
	SK-MEL-28	99.89	0.11





Enzyme Inhibition Kinetics



Nitrocefin (yellow)

Hydrolyzed product (red)

Final concentration & volume of Enzyme (TEM-1) = 3 µL (0.45 nM),
Substrate (NCF) = 12 uL (100 µM)
0.1 % BSA in MOPS buffer = 562 µL (0.02 mM, pH – 7.5)
Inhibitor (in 3 % ACN) = 20 µL (500 µM)

Table 2: Residual Activity and Percent Inhibition of TEM-1 for 3 minutes, 30°C Utilizing Potential Synthesized Inhibitors*

Compound	Molecular Weight (g/mol)	Initial Rate $V_\circ \pm$ SD (ΔA, sec^{-1}) $\times 10^{-3}$	Initial Rate +Inhibitor $V_i \pm$ SD (ΔA, sec^{-1}) $\times 10^{-3}$	Residual Activity (%)	% Inhibition
2a	268.31	1.6870 ± 0.01531	1.2930 ± 0.03163	76.47	23.53
2b	254.28	2.9367 ± 0.26697	2.1840 ± 0.34975	74.37	25.63
2C	280.00	1.159 ± 0.01139	1.0139 ± 0.02758	87.48	12.52
3	252.29	2.0411 ± 0.01252	1.2465 ± 0.01698	61.07	38.93
3 (P99)	252.29	7.1434 ± 0.15520	5.5067 ± 0.15981	77.09	22.91

Conclusion

- In this work, oxadiazolidinone derivatives (**2a**, **2b**, **2c,** and **3**), were prepared using commercially available isocyanate derivatives with synthesized nitrone **1a** and **1b**.The synthesized inhibitors were characterized using ^{1}H and ^{13}C NMR, GC-MS, and IR.

- Afterwards, there were tested against TEM-1 and P99 serine β-lactamase. Compound **2a**, **2b**, **2c,** and **3** showed inhibition ranging from 12-38% and **3** showed 22% inhibition against P99

- MTT Essay was used to test the in vitro cytotoxicity of oxadiazolidinone **2a** on cancer cell lines. **2a** had more activity on renal cancer, decreasing the cell viability of 786-0 by about 18%. The activity on other cell lines ranged from 4-14%.

Acknowledgement

- God almighty,
- My parents,
- Dr. Abbas G. Shilabin,
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- Department of Chemistry and the School of Graduate Studies at ETSU.